The 5-HT2A/1A agonist psilocybin disrupts modal object completion associated with visual hallucinations

Kometer, M ; Cahn, B R ; Andel, D ; Carter, O L ; Vollenweider, F X

Abstract: BACKGROUND:: Recent findings suggest that the serotonergic system and particularly the 5-HT2A/1A receptors are implicated in visual processing and possibly the pathophysiology of visual disturbances including hallucinations in schizophrenia and Parkinson's disease. METHODS:: To investigate the role of 5-HT2A/1A receptors in visual processing the effect of the hallucinogenic 5-HT2A/1A agonist psilocybin (125 and 250 g/kg vs. placebo) on the spatiotemporal dynamics of modal object completion was assessed in normal volunteers (n = 17) using visual evoked potential recordings in conjunction with topographic-mapping and source analysis. These effects were then considered in relation to the subjective intensity of psilocybin-induced visual hallucinations quantified by psychometric measurement. RESULTS:: Psilocybin dose-dependently decreased the N170 and, in contrast, slightly enhanced the P1 component selectively over occipital electrode sites. The decrease of the N170 was most apparent during the processing of incomplete object figures. Moreover, during the time period of the N170, the overall reduction of the activation in the right extrastriate and posterior parietal areas correlated positively with the intensity of visual hallucinations. CONCLUSIONS:: These results suggest a central role of the 5-HT2A/1A-receptors in the modulation of visual processing. Specifically, a reduced N170 component was identified as potentially reflecting a key process of 5-HT2A/1A receptor-mediated visual hallucinations and aberrant modal object completion potential.

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Kometer Michael, Cahn Baruch R., Andel David, Carter Olivia L., Vollenweider Franz X.

Abstract

Background: Recent findings suggest that the serotonergic system and particularly the 5-HT2A/1A receptors are implicated in visual processing and possibly the pathophysiology of visual disturbances including hallucinations in schizophrenia and Parkinson's disease.

Methods: To investigate the role of 5-HT2A/1A receptors in visual processing the effect of the hallucinogenic 5-HT2A/1A agonist psilocybin (125 and 250 μg/kg vs placebo) on the spatiotemporal dynamics of modal object completion was assessed in normal volunteers (n=17) using visual evoked potential (VEP) recordings in conjunction with topographic-mapping and source-analysis. These effects were then considered in relation to the subjective intensity of psilocybin-induced visual hallucinations quantified by psychometric measurement.

Results: Psilocybin dose-dependently decreased the N170 and, in contrast, slightly enhanced the P1 component selectively over occipital electrode sites. The decrease of the N170 was most apparent during the processing of incomplete object figures. Moreover, during the time period of the N170 the overall reduction of the activation in the right extrastriate and posterior parietal areas correlated positively with the intensity of visual hallucinations.

Conclusions: These results suggest a central role of the 5-HT2A/1A-receptors in the modulation of visual processing. Specifically, a reduced N170 component was identified as potentially reflecting a key process of 5-HT2A/1A receptor–mediated visual hallucinations and aberrant modal object completion potential.

Keywords: hallucinogen; serotonin; 5-HT2A; N170; P1; visual hallucination

Introduction

Despite the fact that the visual and serotonergic systems are among the most explored research topics in neuroscience, there is sparse information about the role of serotonin (5-HT) in visual processing. However, the high expression of the 5-HT1A (1,2) and 5-HT2A receptors (3-5) in the visual areas V1, V2, V3 and lateral suprasylvian cortex (LS) suggest a central role of the 5-HT1A and 2A receptors in visual processing. Furthermore, 5-HT2A receptors have been implicated in the pathogenesis of visual hallucinations in Parkinson's (6-8) and schizophrenic patients (9). In support of this view, visual hallucinations in Parkinson's patients have been associated with increased density of 5-HT2A receptors in cortical visual pathways (6,8) and effectively treated with the 5-HT2A receptor inverse agonist pimavanserin (7). Moreover, visual disturbances and hallucinations induced by the indoleamine hallucinogen psilocybin are predominantly mediated by the 5-HT2A receptor (10) and resemble, in phenomenological (11) and behavioural measurements (12), visual disturbances found in schizophrenia (13,14). Hence, there is an increasingly recognized role for the 5-HT2A receptors in the pathogenesis of visual hallucinations, while the brain mechanisms mediating between 5-HT2A receptor activation and visual hallucination are still unknown.

To further characterize the role of the 5-HT2A/1A receptors in visual processing and to elucidate possible pathophysiological mechanisms of visual deficits and hallucinations, we assessed the effect of the mixed 5-HT2A/1A agonist psilocybin (125 μg/ and 250 μg/kg vs. placebo) in healthy volunteers. Electroencephalographic recordings were made while participants viewed Kanizsa- and non-Kanizsa-figures (Figure 1). Behavioural responses were recorded and high-density electrical mapping with source-analysis was used. This allowed for measurement of the spatiotemporal brain dynamics of visual modal object completion and its association with the appearance of visual hallucinations.

Modal object completion refers to the illusory perception of object boundaries and their enclosing surface in the absence of any direct sensory information depicting these boundaries or surfaces. The brain's ability to interpolate the existence of object surface boundaries is essential for accurate object recognition in situations where only ambiguous or incomplete retinal information of the object is available (i.e. due to partial occlusion or poor illumination). Modal completion has been of particular interest to vision scientists as it is critical for the accurate perception of objects and the delineation of multiple objects from themselves or their

background. Imaging studies provide strong evidence that the intermediate lateral occipital complex (LOC) as well as the early visual area V2 are likely to play a major role in modal completion (15-18), while the involvement of the primary visual cortex is more contentious (19). Electrophysiological studies revealed that modal object completion of simple figures such as Kanizsa-figures is predominantly indexed by the modulation of the N170 component (20-25), which appears to be driven primarily by the two critical processes underlying modal object completion, boundary-completion (22) and region-based-segmentation (25). In some previous studies the presentation of Kanizsa-figures, compared to control figures, have additionally been associated with an enhancement of the earlier P1 (26) and the subsequent closure negativity (N_{cl}) component (22). However, as the N_{cl} component is more reliably induced by more complex fragmented images and reflects successful recognition of complex images (27,28) it is unlikely to be as relevant to the processing of the simple Kanizsa-figures used in the current experiment.

The high density of 5-HT1A (1,2) and 5-HT2A (3-5) receptors in LOC and V2, as well as the strong activation seen in these areas after psilocybin administration during resting state (29) suggest that psilocybin might influence modal object completion. As these processes are so critical in defining one's perceptual experience, this study aims to characterize and understand the means by which 5-HT2A/1A receptor activity can alter this fundamental aspect of visual experience.

Methods and Materials

Subjects

Healthy right-handed subjects (8 males, 9 females, mean age 28.8 +/- 3.5 years) were recruited through advertisement from the University of Zürich. All subjects were healthy according to physical examination including electrocardiography, hematogram and detailed blood analysis. The DIA-X diagnostic expert system (30) and a clinical interview was used to exclude subjects with present or antecedent psychiatric disorders, regular alcohol or substance abuse and a history of major psychiatric disorders in first degree relatives. The screening procedure was supplemented by the Freiburg Personality Inventory FPI (31) and Hopkins Symptom Checklist SCL-90-R (32) and a self made substance consumption questionnaire. Since the personality trait factor "emotional lability" was identified to be a predictor for negative experiences during

hallucinogen-induced altered states of consciousness (33), scores exceeding the mean value of normative FPI data by two SD were also used as exclusion criterion. Twelve participants reported having previous experience with psilocybin or other hallucinogens (mean life time experiences 5.5 +/- 4.7 times). None of the subjects consumed any psychoactive substance except alcohol, nicotine, cannabis and caffeine more than once a year.

After being informed by a written and oral description of the procedures of the study and the effects and possible risks of PY administration, all volunteers gave written informed consent to participate in this study. The study was approved by the ethics committee of the University Hospital of Psychiatry, Zurich, and the use of psilocybin in humans was authorized by the Swiss Federal Office for public health.

Substance and dosing

Psilocybin was obtained through the Swiss Federal Office for Public Health. The psilocybin high-dose (HD, 250 mg/kg), low-dose (LD, 125 mg/kg), and lactose placebo (PL) were administered in gelatine capsules of identical number and appearance. On three experimental days, separated from each other by at least two weeks, the subjects came to the lab at 9 am and confirmed they had not eaten breakfast or taken caffeine that morning. In a cross-over randomised design, all subjects received placebo and the two graded doses of psilocybin.

Stimulus and Procedure

The Kanizsa experiment started 120 min post-drug ingestion. During the experiment Kanizsa-figures and control figures were presented at a distance of 1 meter from the participants while they remained fixated on a central fixation-cross. All stimuli consisted of three “pacmen”, each composed of a black circle with a sector of 60 degrees removed. The removed sectors were either aligned such that the stimulus types induced the perception of an illusory triangle or they were rotated by 180 degrees so as to no longer induce the illusory triangle percept (‘Kanizsa’ and ‘non-Kanizsa’ conditions respectively; see Figure 1). The stimuli subtended a visual angle of 3.7° and the support ratio, the ratio of the inducing length to the total length of one illusory contour of the triangle, was 1:2. In order to reduce habituation, the stimuli were presented in four different orientations of equal number, rotated 0°, 90°, 180°, and 270°.

A total of 88 Kanizsa-figures and 88 non-Kanizsa-figures were presented with a stimulus onset asynchrony (SOA) of 3 seconds in a pseudorandomized order in two blocks consisting of 44 Kanizsa-figures and 44 non-Kanizsa-figures each. Participants were required to respond, as quickly as possible, with a button press according to whether or not they saw an illusory triangle on each trial. The response finger was counterbalanced across the subjects to control for faster reaction times of index finger compared to middle finger of the dominant hand. Stimuli remained on the monitor for 300 msec after button-press or for a maximum of 2000 msec.

Psychometry

The Altered States of Consciousness (5D-ASC) rating scale (34) was used to asses the subjective effects of placebo and psilocybin with a primary focus on visual alterations and hallucinations. The 5D-ASC is a visual-analogue scale consisting of 94 items that measure etiology independent alterations in consciousness, including changes in mood, perception, cognition and experience of self and of the environment. The five main factors are: (1) Oceanic Boundlessness, measuring derealization and depersonalization accompanied by changes in affect ranging from heightened mood to euphoria, and alterations in the sense of time. (2) Anxious Ego Dissolution measuring ego-disintegration associated with loss of self-control, thought disorder, arousal, and anxiety. (3) Visionary Restructuralization (VR) assessing visual illusions and (pseudo-) hallucinations, which includes the whole spectrum ranging from elementary to complex hallucinations. (4) Auditory Alterations (AA) comprising auditory illusions and (pseudo-) hallucinations. (5) Reduction of Vigilance assessing changes in vigilance and alertness. The results of the 5D-ASC data are given as percentage scores of maximum absolute scale values.

EEG recording

EEG recordings were made using BioSemi ActiveTwo electrode system with 64 scalp electrodes. Additional electrodes were attached on the outer canthus of each eye to record the horizontal electroocculogram (EOG) and infraorbitally and supraorbitally to the left eye to record the vertical EOG. Electrophysiological signals were band-pass filtered at 0.01-67 Hz and digitized at 256 Hz.

EEG analysis

EEG data were recalculated offline against average reference and band-pass filtered at 1-40 Hz. Correct response trials were segmented from -150 to +600 ms relative to stimulus presentation. To avoid eye movement and other artifacts in further analysis, epochs exceeding ±100 μV in any channel were excluded. The mean +/- SD number of accepted epochs per condition was 85.0 +/- 5.4 for Kanizsa placebo, 85.0 +/- 3.0 for non-Kanizsa placebo, 82.9 +/- 4.6 for Kanizsa low-dose, 83.2 +/- 3.7 for non-Kanizsa low-dose, 78.8 +/- 9.4 for Kanizsa high-dose, 79.4 +/- 9.8 for non-Kanizsa high-dose. These epochs were averaged time-locked to Kanizsa- and non-Kanizsa-figure presentation time to compute the VEP. The 600 ms poststimulus period of the VEP underwent three different analyses to reveal the VEP waveforms, the scalp topography and the source localization. This combination of analysis methods has been increasingly used during the last years, as it reduces experimenter bias associated with the selection of the appropriate time window for statistical analysis of the components (35).

Waveform analysis: To quantify waveform modulations, five symmetrical pairs of electrodes were selected over the maxima of the scalp topographies for the P1 and N170 components (PO7/PO8, PO3/PO4, O1/O2, P1/P2, P3/P4). The specific time windows used for calculation of the mean amplitude (versus the baseline) were determined by the topographic analysis described below. The mean amplitudes of each of these time windows were then subjected to separate repeated measures analysis of variance (ANOVA) with the within-subject factors dose (PL, LD, HD) electrode (PO7/PO8, PO3/PO4, O1/O2, P1/P2, P3/P4), hemiscalp (left, right) and stimulus (Kanizsa, non-Kanizsa).

Topographic analysis: First a spatial k-means cluster analysis identified the predominant topographies (also called template map) appearing in the normalized group-averaged ERPs as a function of time and experimental condition (36). This analysis was constrained by the temporal criterion, that certain topographies must be observed for at least 5 consecutive data points (> 20 ms at a 256 Hz sampling rate). The optimal number of topographies that explained the whole dataset was determined by a modified cross-validation criterion (36). In a second step we statistically verified that maps identified at the group-averaged level also appeared on the individual subject level. Therefore, at each time point the topography of the individual subjects' ERP was compared by means of strength-independent spatial correlation to all template maps and was labeled according to the one with which it best correlated (35,37). This revealed values of

relative map presence (in milliseconds), which were then subjected to a repeated measures ANOVA using stimulus condition, map and dose as within-subject factors.

Source Localization: Standardized low-resolution electromagnetic tomography (sLORETA) was used to estimate the three dimensional intra-cerebral current density distributions underlying the ERPs within the time frames defined by topographic analysis. sLORETA offers a solution of the inverse problem by assuming that the smoothest of all possible activity is the most plausible one (38). The solution space of sLORETA (i.e. the lead field matrix) includes 6239 voxels and was computed with a three-shell spherical head model registered to the neuroanatomical atlas of Talairach and Tournoux (Brain Imaging Centre, Montreal Neurological Institute).

Correlational analyses were performed to determine whether psilocybin-induced changes in the current source density within the time frames defined by topographic analysis were related to visual or auditory hallucinations. First, the differences of the current source density between psilocybin conditions (low- and high-dose) and placebo were calculated separately. In a second step these data were pooled and the product-moment correlations between these differences and the VR and AA scores, respectively, were calculated for each voxel. Significant correlations were identified using nonparametric permutation testing (39) that determined the critical probability threshold values for the observed r-values with correction for multiple testing.

Results

Psychometrics

The subjective effects of psilocybin were assessed by the 5D-ASC rating scale. Repeated measures ANOVA revealed a significant main effect of dose ($F(2,32)=36.596$, $p<0.00001$, $\eta^2=0.70$), factor ($F(4,64)=12.924$, $p<0.00001$, $\eta^2=0.45$) and dose x factor interaction ($F(8,128)=7.09$, $p<0.00001$, $\eta^2=0.31$) . Bonferroni-corrected post-hoc analysis on the VR factor, which was of primary interest in regard to the current study, indicated a significant increase between placebo and low-dose psilocybin ($p<0.00001$) as well as placebo and high-dose psilocybin ($p<0.00001$) but not low-dose and high-dose psilocybin ($p=0.178$). Bonferroni-corrected post-hoc analysis on the AA factor revealed a significant increase between placebo and high-dose psilocybin ($p<0.05$) but not placebo and low-dose psilocybin ($p=1.0$).

Behavioral data

Reaction time was dose-dependently increased by psilocybin ($F(2,32)=18.841$, $p<0.00001$, $\eta^2=0.54$) and was generally faster for Kanizsa- compared to non-Kanizsa-figure ($F(1,16)=36.037$, $p<0.0001$, $\eta^2=0.69$) (Table 1). While psilocybin administration did lead to a dose-dependent increase in error rates ($F(2,32)=3.3291$, $p<0.05$, $\eta^2=0.17$), the error rates remained very low in all conditions (0.88% for placebo, 1.18% for low-dose, 1.92% for high-dose). We are therefore confident that participants were able to do the task under all drug conditions.

Electrophysiological data

Under all 3 drug conditions both stimuli elicited prominent VEP, including the P100 and N170 component (Figure 1) with a maximum over lateral occipital scalp areas (Figure 3). The subsequent topographic analysis detected nine different scalp topographies, which accounted for the 600 ms post-stimulus periods across all conditions with a global explained variance (GEV) of 96.74 %. The first three time periods of stable scalp topographies were identical across conditions lasting from 0-86 ms, 90-144 ms, 148-223 ms (Figure S1). These time periods were used to define the time window for quantifying the P1 and N170 VEP in a more objective manner (Murray et al., 2008) and for the subsequent source localization and correlation analysis (Analysis referring to the P300 component can be found in supplementary material).

P1

During the time-range of the P1 component (90-144 ms) a 3 x 2 x 5 x 2 repeated measures ANOVA (using dose, hemiscalp, electrode, and stimulus as within factors) revealed only a main effect of electrode ($F(4, 64)=33.42$, $p<0.00001$, $\eta^2=0.68$) and a significant interaction of electrode and dose ($F(8, 128)=3.43$, $P<0.01$, $\eta^2=0.18$). Performing bonferroni-corrected post-hoc analysis of this interaction, the P1 amplitude was found to be significantly increased from placebo only at the O1/O2 electrodes by low dose ($p < 0.05$) and high dose ($p < 0.0001$) psilocybin. No effect of psilocybin was observed at other electrodes, nor did any other interaction reach significance. This result indicates that the effect of psilocybin on the P1 component is locally restricted to occipital electrode sites and is independent of hemiscalp and stimulus condition (Figure 2). Furthermore, neither the main effect for dose ($F(2,32)=0.95$, $p=0.39$, $\eta_2=0.06$), stimulus ($F(1,16)=0.77$, $p=0.39$, $\eta_2=0.05$) nor hemiscalp ($F(1,16)=1.71$, $p=0.21$, $\eta_2=0.10$) reached significance.

To further elucidate topographic modulation of the P1 component, the spatial correlation procedure was used first to assess the number of time frames a given topography from the group-averaged data was present in a given condition across subjects (see methods). These values were subjected to a repeated measures ANOVA (using dose, stimulus, and map as within factors), which provided no evidence of topographic specificity to one stimulus or dose condition (all P's > 0.15).

Source estimation from the 90-144 ms period yielded activity within LOC and V2 in both hemispheres across all conditions. The psilocybin-induced increase in the P1 component over O1/O2 electrode sites seen in the VEP waveform-analysis was localized most strongly in V2 and extended to LOC and V1 in the right hemisphere in the high-dose condition. The psilocybin induced increase in the source-density did neither correlate with visual (all P's > 0.17) nor with auditory hallucinations (all P's > 0.98).

N170

To quantify the modulation of the N170 component, area measurements over the 148-223 ms period were subjected to a 3 x 2 x 5 x 2 repeated measures ANOVA (using dose, hemiscalp, electrode, and stimulus as within factors). Psilocybin dose-dependently decreased the N170 amplitude ($F(2,32)=17.170$, $p<0.00001$, $\eta^2=0.52$) and bonferroni-corrected post-hoc analysis revealed that the magnitude differences were significant between all dose conditions (Figure 2). The significant interaction between dose and hemiscalp ($F(2,32)=5.30$, $p<0.05$, $\eta^2=0.25$) and the visual inspection of the VEP indicated a stronger psilocybin-induced decrease over the right compared to the left hemiscalp. In accordance with numerous previous studies (20-25) a significant main effect of the stimulus condition was observed ($F(1,16)=35.863$, $p<0.0001$, $\eta^2=0.70$), with a greater magnitude in the Kanizsa- compared to the non-Kanizsa-condition. This magnitude difference between Kanizsa- and non-Kanizsa conditions dose-dependently decreased as revealed by the significant interaction between dose and condition ($F(2,32)=3.4485$, $p<0.05$, $\eta^2=0.18$).

The topographic pattern analysis of the collective data indicated that the effects of strength modulation in the electrical field were not associated with topographic modulations. This was subsequently confirmed by subjecting individual subject data to repeated measures ANOVA (all P's > 0.25).

The source estimation revealed activity within LOC and V2 in both hemispheres in all conditions (Figure 3). Current source density was stronger within right-lateralized LOC and V2 in the Kanizsa- compared to non-Kanizsa-condition. Psilocybin dose-dependently decreased the differential activation of the two stimulus conditions and reduced the current source density within LOC, V2 and fusiform gyrus in both stimulus conditions. The psilocybin-induced current source density reduction over right-lateralized LOC, V2 and posterior parietal areas correlated significantly with the increased intensity of visual hallucinations (Figure 4), while no correlation could be revealed with auditory hallucinations (all Ps > 0.64).

Discussion

The present investigation revealed three main findings. First, the data indicate that the mixed 5-HT2A/1A-receptor agonist psilocybin distinctively modulates the two early visual processing components. Specifically, while we found a strong dose-dependent decrease of the N170 component (148-223 ms post-stimulus) the earlier visual P1 component (90-144 ms post-stimulus) was slightly increased over occipital electrode sites. Second, the reduction of the N170 component was stronger for the Kanizsa-figure condition as compared to the non-Kanizsa condition. Third, during this time range the decrease in activation over right-lateralized extrastriate and posterior parietal cortex correlated with the reported intensity of visual hallucinations.

The time frame of the N170 component has been regarded as a critical period for object completion based on numerous findings of enhanced N170 amplitude and underlying LOC and V2 activation evoked by Kanizsa- compared to control figures (20-25). The preferential reduction of the N170 amplitude in Kanizsa compared to non-Kanizsa condition by psilocybin indicates a central role of the 5-HT2A/1A receptors in object-completion. Since two sub-processes of object completion, i.e. boundary completion (22) and region-based segmentation (25), have been shown to underlay the enhanced N170 amplitude in the Kanizsa-condition, it would be interesting to further investigate which of these two sub-processes is modulated by signal transmission at 5-HT2A/1A receptors.

The finding that psilocybin slightly enhanced P1 amplitude may reflect a generalized effect of 5-HT2A/1A mediated increase in perceived brightness which is often reported after indolamine hallucinogen administration (40). This is supported by previous studies using

comparable Kanizsa-figures showing the P1 component to be sensitive to stimulus brightness (41) as well as eccentricity (20) and symmetry (41).

Given that psilocybin strongly decreased the N170 amplitude in both the Kanizsa and the non-Kanizsa conditions, we suggest that it modulates additional processes beyond proper object completion. Disruption of these processes might play a role in visual hallucinations, since their reported intensity was significantly correlated with the 3-dimensional current source density reduction over right-lateralized LOC, V2 and posterior parietal areas during the time-frame of the N170 component. This localization is in agreement with previous imaging studies reporting decreased extrastriate activation in response to external visual stimuli in patients with visual hallucinations compared to patients without hallucinations (42-44). Furthermore, we found decreased activation specifically during the time-range of the N170 component. Decreased activation during this time range has been previously reported in studies investigating acoustic (45,46) and visual hallucinations (23). We therefore suggest that decreased stimulus-induced activation in modality-specific cortical areas during the time range of the N1/ N170 component may be a characteristic of acoustic as well as visual hallucinations. This relationship between the increased intensity of visual hallucinations and the decreased stimulus-evoked activation of extrastriate areas during the time-range of the N170 component could reflect a competition for neuronal resources in the processing of internally (hallucination) and externally (sensory-driven) generated information (46,47). A comparable mechanism has previously been suggested to be relevant in the formation of acoustic hallucinations (48). Increased extrastriate activation which could interfere with the processing of external stimuli, has been observed previously during visual hallucinations in the absence of external stimulation in different psychiatric disorders (43,49-51) as well as after psilocybin administration (29). In line with this interpretation we additionally found a correlation between reduced posterior parietal activation and hallucinatory severity. The posterior parietal cortex controls attention to visual stimuli by modulating visual cortex activity (52) during the time-range of the N170 component (53). The reduced posterior parietal activation might therefore reflect a psilocybin-induced failure to allocate attention and thus neuronal resources to external stimuli.

Importantly, we identified reduced extrastriate visual cortex activation during the time range of the N170 as a potential key component of 5-HT2A/1A agonist-induced visual hallucinations. Such a mechanism could also underlie the visual disturbances and hallucinations

reported in Parkinson and schizophrenia patients, since increased 5-HT2A receptor densities have been found in these conditions and associated with visual hallucinations (6,8,9). In support of this notion, Parkinson's patients with visual hallucinations showed more pronounced impairments in visual object recognition (54,55) and more prominent reductions in extrastriate cortex activation to visual stimuli than patients without hallucinations (44). Moreover, a reduction of the N170 component has also been reported in schizophrenia patients and associated with visual hallucinations using Kanizsa-figures (23). These observations are in line with our finding of a correlation between reduced extrastriate activation during the time range of the N170 component and the intensity of visual hallucinations. It is noteworthy, however, that two other previous studies using Kanizsa-figures in schizophrenia reported either no reduction (24) or only a reduction at a trend level (56). The apparent discrepancy between these results and our findings may be due to the fact that only about 30% of schizophrenia patients typically report visual hallucinations (57) and many of the patients tested were medicated with atypical antipsychotics with 5-HT2A antagonistic activity. It might, therefore be possible that a disrupted 5-HT2A receptor system may be relevant only to a subgroup of schizophrenia patients. Given that we did not find a decrease of the P1 component after psilocybin administration and the robust reduction of the P1 component reported in schizophrenia (23,24,56,58) was not associated with psychotic symptoms (58) suggests that the reduction of the P1 component in schizophrenia is not related to serotonergic alterations and may rather represent an endophenotype of the schizophrenia spectrum (58,59).

Since psilocybin is a mixed 5-HT2A/1A agonist (60) resulting in downstream effects on the dopamine systems (61), the exact pharmacological mechanisms of the observed psilocybin effects require further investigation. However, converging lines of evidence indicate that psilocybin's psychotomimetic effects are mediated through the 5-HT2A-receptor activation (10,62). For example, behavioral effects of psilocybin are lacking in 5-HT2A receptor knockout mice (63) and in humans nearly all psychotomimetic effects including visual distortions and hallucinations can be blocked by the 5-HT2A antagonist ketanserin (64,65), but not by blocking downstream dopaminergic effects with haloperidol (64). These findings, in combination with evidence regarding the importance of the 5-HT2A receptors in the pathophysiology of visual hallucinations (6-9), indicate that the reported effects of psilocybin are likely to be mediated by 5-HT2A receptor activation. However, given that 5-HT1A receptors additionally influence the

psychotomimetic effects of indolamine hallucinogens (66) and 5-HT1A receptors are widespread in the visual cortex (1,2), further studies are needed to exclude an additional contribution of 5-HT1A receptor on the spatiotemporal dynamics of visual object completion and hallucinations.

Figure Legends

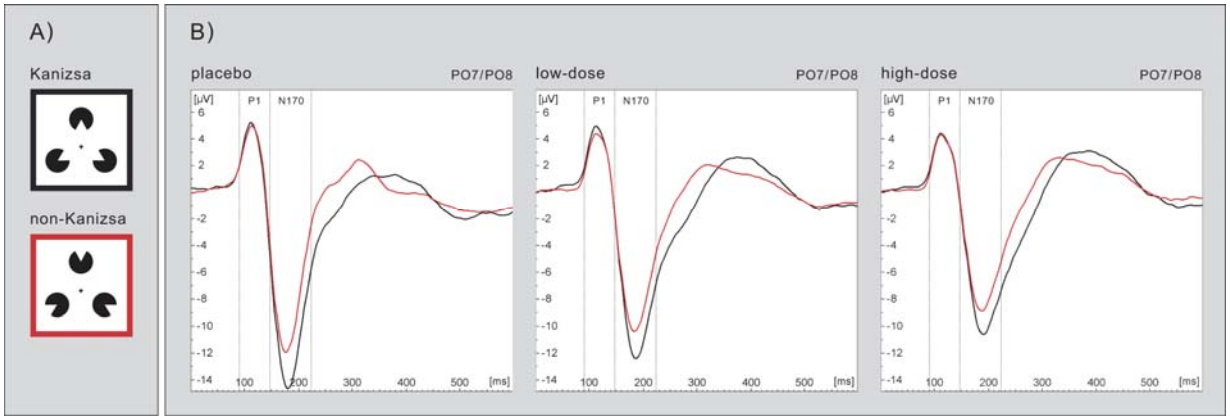

Figure 1

A) Example of Kanizsa-figure and non-Kanizsa-figure. B) Group-averaged VEP waveforms in response to Kanizsa-figures (black traces) and non-Kanizsa-figures (red traces) for placebo, low-dose and high-dose condition averaged from the left and right posterior electrode sites PO7/PO8, where P1 and N170 amplitude was most pronounced. Dashed lines indicate the time period of stable topographic configuration of the P1 and N170 component.

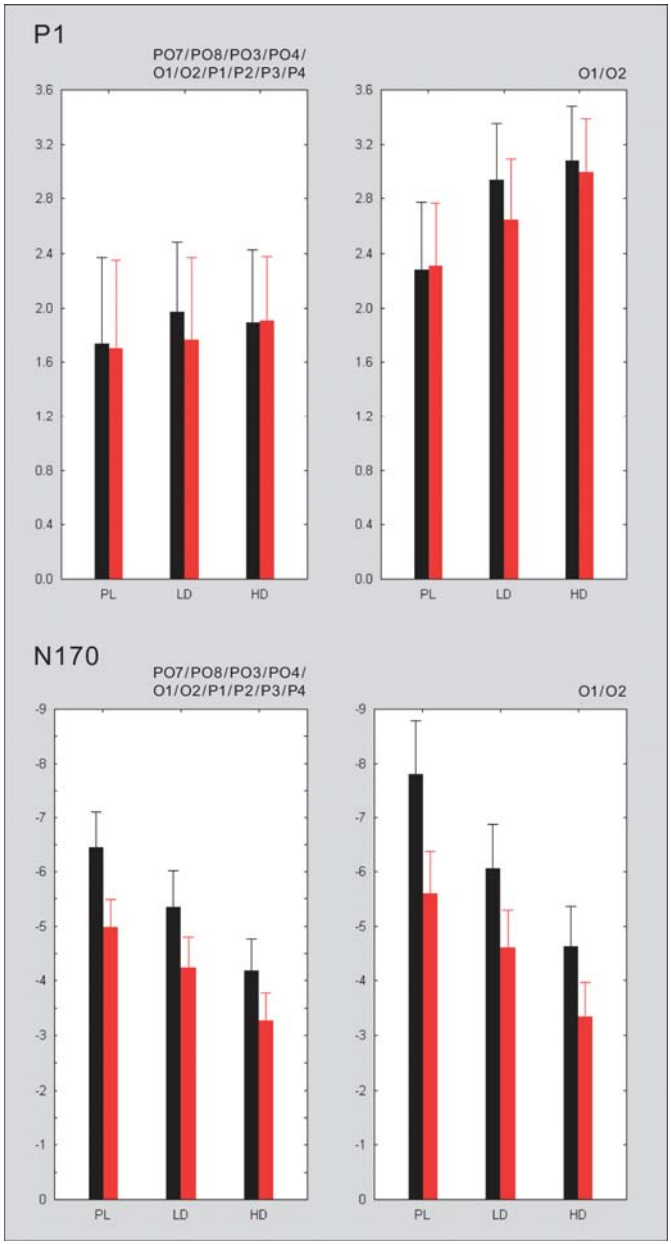

Figure 2

The bar graphs display mean areas of the P1 (top) and N170 (bottom) components measured from 10 parieto-occipital electrodes sites (left) and from occipital electrode sites O1/O2 (right) symmetrically localized over both hemiscalps (see Methods and Materials for details). Mean areas are displayed for Kanizsa-figures (black) and non-Kanizsa-figures (red) for all dose conditions (PL = placebo, LD = low-dose, HD = high-dose). Vertical bars denote standard deviations. The y axis presents amplitude in microvolts (µV).

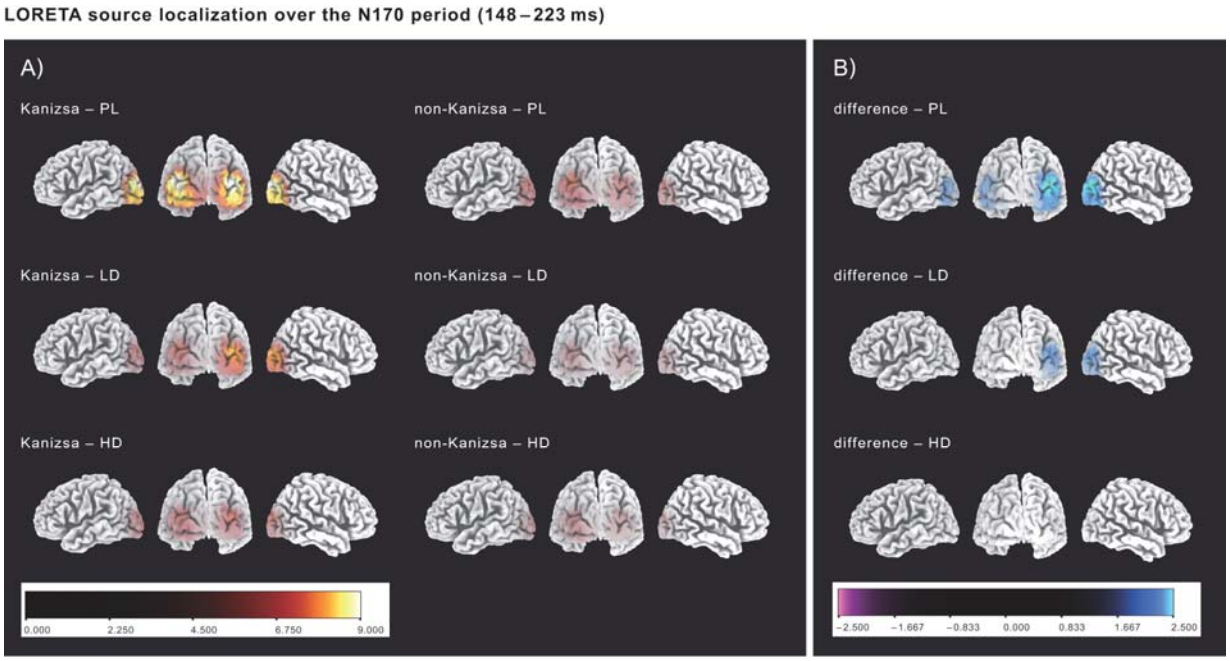

Figure 3

A) Group-averaged LORETA source estimations for each stimulus (Kanizsa, non-Kanizsa) and dose (PL = placebo, LD = low-dose, HD = high-dose) conditions over the N170 period (148-223 ms). B) Group-averaged differences of the source estimations between Kanizisa and non-Kanizsa condition.

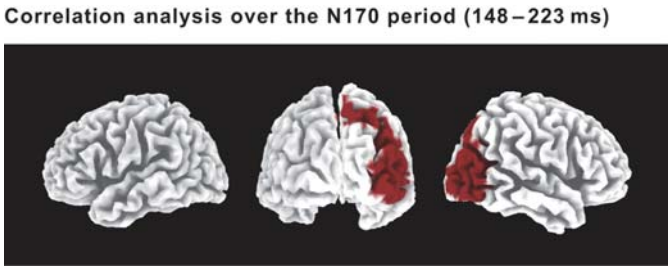

Figure 4

The voxel-wise correlation between visual hallucinations and placebo - psilocybin current source density difference revealed that psilocybin-induced decrease in current source density in right extrastriate areas and posterior parietal areas over the N170 period (148-223 ms) positively correlated with the intensity of visual hallucinations (significant areas $p < 0.05$ are shown in red). 194 voxels reached 0.05 significance criterion with a mean r value of 0.57.

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References

1. Dyck RH, Cynader MS (1993): Autoradiographic localization of serotonin receptor subtypes in cat visual cortex: transient regional, laminar, and columnar distributions during postnatal development. *J Neurosci* 13: 4316-4338.

2. Gerstl F, Windischberger C, Mitterhauser M, Wadsak W, Holik A, Kletter K et al (2008): Multimodal imaging of human early visual cortex by combining functional and molecular measurements with fMRI and PET. *Neuroimage* 41: 204-211.

3. Jakab RL, Goldman-Rakic PS (1998): 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: Possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc Natl Acad Sci* 95: 735-740.

4. Adams KH, Pinborg LH, Svarer C, Hasselbalch SG, Holm S, Haugbol S et al (2004): A database of (18F)-altanserin binding to 5-HT2A receptors in normal volunteers: normative data and relationship to physiological and demographic variables. *Neuroimage* 21: 1105-1113.

5. Watakabe A, Komatsu Y, Sadakane O, Shimegi S, Takahata T, Higo N et al (2009): Enriched expression of serotonin 1B and 2A receptor genes in macaque visual cortex and their bidirectional modulatory effects on neuronal responses. *Cereb Cortex* 19: 1915-1928.

6. Ballanger B, Strafella AP, van ET, Zurowski M, Rusjan PM, Houle S et al (2010): Serotonin 2A receptors and visual hallucinations in Parkinson disease. *Arch Neurol* 67: 416-421.

7. Meltzer HY, Mills R, Revell S, Williams H, Johnson A, Bahr D et al (2010): Pimavanserin, a serotonin(2A) receptor inverse agonist, for the treatment of parkinson's disease psychosis. *Neuropsychopharmacology* 35: 881-892.

8. Huot P, Johnston TH, Darr T, Hazrati LN, Visanji NP, Pires D et al (2010): Increased 5-HT2A receptors in the temporal cortex of parkinsonian patients with visual hallucinations. *Mov Disord* 25: 1399-1408.

9. Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF et al (2008): Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* 452: 93-97.

10. Geyer MA, Vollenweider FX (2008): Serotonin research: contributions to understanding psychoses. *Trends Pharmacol Sci* 29: 445-453.

11. Vollenweider FX, Geyer MA (2001): A systems model of altered consciousness: Integrating natural and drug-induced psychoses. *Brain Res Bull* 56: 495-507.

12. Carter OL, Pettigrew JD, Burr DC, Alais D, Hasler F, Vollenweider FX (2004): Psilocybin impairs high-level but not low-level motion perception. *NeuroReport* 15: 1947-1951.

13. Chen Y, Levy DL, Sheremata S, Holzman PS (2004): Compromised late-stage motion processing in schizophrenia. *Biol Psychiatry* 55: 834-841.

14. Chen Y, Nakayama K, Levy D, Matthysse S, Holzman P (2003): Processing of global, but not local, motion direction is deficient in schizophrenia. *Schizophr Res* 61: 215-227.

15. Mendola JD, Dale AM, Fischl B, Liu AK, Tootell RB (1999): The representation of illusory and real contours in human cortical visual areas revealed by functional magnetic resonance imaging. *J Neurosci* 19: 8560-8572.

16. Halgren E, Mendola J, Chong CD, Dale AM (2003): Cortical activation to illusory shapes as measured with magnetoencephalography. *Neuroimage* 18: 1001-1009.

17. Stanley DA, Rubin N (2003): fMRI activation in response to illusory contours and salient regions in the human Lateral Occipital Complex. *Neuron* 37: 323-331.

18. Montaser-Kouhsari L, Landy MS, Heeger DJ, Larsson J (2007): Orientation-selective adaptation to illusory contours in human visual cortex. *J Neurosci* 27: 2186-2195.

19. Seghier ML, Vuilleumier P (2006): Functional neuroimaging findings on the human perception of illusory contours. *Neuroscience and Biobehavioral Reviews* 30: 595-612.

20. Murray MM, Wylie GR, Higgins BA, Javitt DC, Schroeder CE, Foxe JJ (2002): The spatiotemporal dynamics of illusory contour processing: Combined high-density electrical mapping, source analysis, and functional magnetic resonance imaging. *J Neurosci* 22: 5055-5073.

21. Murray MM, Foxe DM, Javitt DC, Foxe JJ (2004): Setting boundaries: brain dynamics of modal and amodal illusory shape completion in humans. *J Neurosci* 24: 6898-6903.

22. Murray MM, Imber ML, Javitt DC, Foxe JJ (2006): Boundary completion is automatic and dissociable from shape discrimination. *J Neurosci* 26: 12043-12054.

23. Spencer KM, Nestor PG, Perlmutter R, Niznikiewicz MA, Klump MC, Frumin M et al (2004): Neural synchrony indexes disordered perception and cognition in schizophrenia. *Proc Natl Acad Sci* : 1-6.

24. Foxe JJ, Murray MM, Javitt DC (2005): Filling-in in schizophrenia: A high-density electrical mapping and source-analysis investigation of illusory contour processing. *Cereb Cortex* 15: 1914-1927.

25. Yoshino A, Kawamoto M, Yoshida T, Kobayashi N, Shigemura J, Takahashi Y et al (2006): Activation time course of responses to illusory contours and salient region: A high-density electrical mapping comparison. *Brain Res* 1071: 137-144.

26. Brodeur M, Lepore F, Lepage M, Bacon BA, Jemel B, Debruille JB (2008): Alternative mode of presentation of Kanizsa figures sheds new light on the chronometry of the mechanisms underlying the perception of illusory figures. *Neuropsychologia* 46: 554-566.

27. Doniger GM, Foxe JJ, Murray MM, Higgins BA, Snodgrass JG, Schroeder CE et al (2000): Activation timecourse of ventral visual stream object-recognition areas: high density electrical mapping of perceptual closure processes. *J Cogn Neurosci* 12: 615-621.

28. Sehatpour P, Dias EC, Butler PD, Revheim N, Guilfoyle DN, Foxe JJ et al (2010): Impaired visual object processing across an occipital-frontal-hippocampal brain network in schizophrenia: an integrated neuroimaging study. *Arch Gen Psychiatry* 67: 772-782.

29. Vollenweider FX, Leenders KL, Scharfetter C, Maguire P, Stadelmann O, Angst J (1997): Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. *Neuropsychopharmacology* 16: 357-372.

30. Wittchen,HU, Pfister,H (1997): *DIA-X-Interview* . Frankfurt: Swets Test Services.

31. Fahrenberg,J, Hampel,R, Selg,H (1984): *Das Freiburger Persönlichkeitsinventar FPI* , 4 ed. Göttingen: Hogrefe.

32. Derogatis,LR (1994): *SCL-90-R: Symptom Checklist-90-R. Administration, scoring and procedures manual* , 3rd Edition ed. Minneapolis: National Computer Systesm.

33. Dittrich A (1994): Psychological aspects of altered states of consciousness of the LSD type: measurements of their basic dimensions and prediction of individual differences. In Pletscher A, Ladewig D, editors. *50 Years of LSD. Current Status and Perspectives of Hallucinogens*, 1 ed. New York: Parthenon Publishing, pp 101-118.

34. Dittrich A (1998): The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. *Pharmacopsychiat* 31: 80-84.

35. Murray MM, Brunet D, Michel CM (2008): Topographic ERP analyses: a step-by-step tutorial review. *Brain Topogr* 20: 249-264.

36. Pascual-Marqui RD, Michel CM, Lehmann D (1995): Segmentation of brain electrical activity into microstates: model estimation and validation. *IEEE Trans Biomed Eng* 42: 658-665.

37. Brandeis D, Lehmann D, Michel CM, Mingrone W (1995): Mapping event-related brain potential microstates to sentence endings. *Brain Topogr* 8: 145-159.

38. Pascual-Marqui RD (2002): Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. *Methods Find Exp Clin Pharmacol* 24 Suppl D: 5-12.

39. Nichols TE, Holmes AP (2002): Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp* 15: 1-25.

40. Siegel RK, Jarvik ME (1975): Drug-induced hallucinations in animals and man. In Siegel RK, West LJ, editors. *Hallucinations. Behavior, Experience & Theory*. New York: John Wiley & Sons, pp 81-161.

41. Proverbio AM, Zani A (2002): Electrophysiological indexes of illusory contours perception in humans. *Neuropsychologia* 40: 479-491.

42. Howard R, Williams S, Bullmore E, Brammer M, Mellers J, Woodruff P et al (1995): Cortical response to exogenous visual stimulation during visual hallucinations. *Lancet* 345: 70.

43. Ffytche DH, Howard RJ, Brammer MJ, David A, Woodruff P, Williams S (1998): The anatomy of conscious vision: an fMRI study of visual hallucinations. *Nature Neurosci* 1: 738-742.

44. Meppelink AM, de Jong BM, Renken R, Leenders KL, Cornelissen FW, van LT (2009): Impaired visual processing preceding image recognition in Parkinson's disease patients with visual hallucinations. *Brain* 132: 2980-2993.

45. Tiihonen J, Hari R, Naukkarinen H, Rimon R, Jousmäki V, Kajola M (1992): Modified activity of the human auditory cortex during auditory hallucinations. *Am J Psychiatry* 149: 255-257.

46. Hubl D, Koenig T, Strik WK, Garcia LM, Dierks T (2007): Competition for neuronal resources: how hallucinations make themselves heard. *Br J Psychiatry* 190: 57-62.

47. Allen P, Laroi F, McGuire PK, Aleman A (2008): The hallucinating brain: a review of structural and functional neuroimaging studies of hallucinations. *Neurosci Biobehav Rev* 32: 175-191.

48. Ford JM, Roach BJ, Jorgensen KW, Turner JA, Brown GG, Notestine R et al (2009): Tuning in to the voices: a multisite FMRI study of auditory hallucinations. *Schizophr Bull* 35: 58-66.

49. Silbersweig DA, Stern E, Frith GH, Cahill C, Holmes A, Grootoonk S et al (1995): A functional neuroanatomy of hallucinations in schizophrenia. *Nature* 378: 176-179.

50. Holroyd S, Wooten GF (2006): Preliminary FMRI evidence of visual system dysfunction in Parkinson's disease patients with visual hallucinations. *J Neuropsychiatry Clin Neurosci* 18: 402-404.

51. Oertel V, Rotarska-Jagiela A, van dV, V, Haenschel C, Maurer K, Linden DE (2007): Visual hallucinations in schizophrenia investigated with functional magnetic resonance imaging. *Psychiatry Res* 156: 269-273.

52. Bressler SL, Tang W, Sylvester CM, Shulman GL, Corbetta M (2008): Top-down control of human visual cortex by frontal and parietal cortex in anticipatory visual spatial attention. *J Neurosci* 28: 10056-10061.

53. Rose M, Schmid C, Winzen A, Sommer T, Buchel C (2005): The functional and temporal characteristics of top-down modulation in visual selection. *Cereb Cortex* 15: 1290-1298.

54. Meppelink AM, Koerts J, Borg M, Leenders KL, van LT (2008): Visual object recognition and attention in Parkinson's disease patients with visual hallucinations. *Mov Disord* 23: 1906-1912.

55. Koerts J, Borg MA, Meppelink AM, Leenders KL, van BM, van LT (2010): Attentional and perceptual impairments in Parkinson's disease with visual hallucinations. *Parkinsonism Relat Disord* 16: 270-274.

56. Spencer KM, Nestor PG, Niznikiewicz MA, Salisbury DF, Shenton ME, McCarley RW (2003): Abnormal neural synchrony in schizophrenia. *J Neurosci* 23: 7407-7411.

57. Bracha HS, Wolkowitz OM, Lohr JB, Karson CN, Bigelow LB (1989): High prevalence of visual hallucinations in research subjects with chronic schizophrenia. *Am J Psychiatry* 146: 526-528.

58. Yeap S, Kelly SP, Sehatpour P, Magno E, Garavan H, Thakore JH et al (2008): Visual sensory processing deficits in Schizophrenia and their relationship to disease state. *Eur Arch Psychiatry Clin Neurosci* 258: 305-316.

59. Yeap S, Kelly SP, Sehatpour P, Magno E, Javitt DC, Garavan H et al (2006): Early visual sensory deficits as endophenotypes for schizophrenia: high-density electrical mapping in clinically unaffected first-degree relatives. *Arch Gen Psychiatry* 63: 1180-1188.

60. McKenna DJ, Repke DB, Peroutka SJ (1990): Differential interactions of indolealkylamines with 5-hydroxytrypamine receptor subtypes. *Neuropharmacology* 29: 193-198.

61. Vollenweider,FX, Vontobel,P, Hell,D, and Leenders,KL (1998): 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man - a PET study with [11C] raclopride. *Neuropsychopharmacology* 20: 424-433.

62. Gonzalez-Maeso J, Sealfon SC (2009): Psychedelics and schizophrenia. *Trends Neurosci* 32: 225-232.

63. Gonzalez-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R et al (2007): Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* 53: 439-452.

64. Vollenweider FX, Vollenweider-Scherpenhuyzen MFI, Bäbler A, Vogel H, Hell D (1998): Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *NeuroReport* 9: 3897-3902.

65. Carter OL, Burr DC, Pettigrew JD, Wallis GM, Hasler F, Vollenweider FX (2005): Using psilocybin to investigate the relationship between attention, working memory, and the serotonin 1A and 2A receptors. *J Cogn Neurosci* 17: 1497-1508.

66. Nichols DE (2004): Hallucinogens. *Pharmacol Ther* 101: 131-181.